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DEVELOPMENT OF AN APPARATUS FOR PARTIAL CELL IRRADIATION

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FOREWORD

This report was prepared by the following personnel:

IRA L. MORGAN, Ph.D.*
LOY D. ENGLAND, B.S., B.D.*
ROBERT L. LAUNER, B.A.*
WILLIAM E. TUCKER, B.A., M.A.*
JOSEPH S. PIZZUTO, Captain, USAF, MSC†
CHARLES M. POMERAT, Ph.D.‡

(*Texas Nuclear Corporation, Austin, Texas; †Bionucleonics Department, USAF School of Aerospace Medicine; ‡Pasadena Foundation for Medical Research, Pasadena, California)

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ABSTRACT

An apparatus has been developed which allows 16 Mev proton irradiation of selected areas of single living biologic cells cultured in vitro. The highly collimated proton microbeam passes through the tissue culture and is monitored as to number and energy of protons. Extrapolation of individual proton energies allows determination of the energy loss in the tissue.

Specific advances, over earlier work in partial cell and microbeam irradiation, include the collimation of 16 Mev protons, cellular alignment technic, and special monitoring methods.

This technical documentary report has been reviewed and is approved.

Robert B. Payne

ROBERT B. PAYNE
Colonel, USAF, MSC
Chief, Operations Division

DEVELOPMENT OF AN APPARATUS FOR PARTIAL CELL IRRADIATION

1. INTRODUCTION

Partial cell irradiation (PCI) experimentation was first reported by Tchakhotine (7) in 1912. He obtained a 2800 A. microbeam from a magnesium spark by means of a prism and a refracting lens. Microscopic observations were made during the alignment procedure and during bombardment by ultraviolet radiation, which dominated the field of PCI until Kirkpatrick and Pattee (3) demonstrated in 1953 that x-rays could be focused to a small spot. They, however, did not report PCI. C. L. Smith (6) used a metal collimator instead of the focusing method of Kirkpatrick and Pattee in an attempt to obtain an x-ray microbeam, but the microbeam intensity proved insufficient.

Other investigations used micro sources inside the target cell employing chemical injection or mechanical injection of the radioactive material. McQuade et al. (5) localized radioactive thymidine in chromosomes and in nuclei by chemical methods—that is, by mixing radioactive thymidine in the culture medium. T. R. Munro (4) placed polonium-210 on the tip of a needle and injected it into part of the cytoplasm. The nuclear region was untouched by the alpha particles emitted by the Po^{210}. Although yielding much useful information, these methods have been difficult because of dose considerations or because of the low damaging power of the resulting microbeam. Provided suitable collimation could be developed, it seemed that heavy charged particles from an accelerator would be more acceptable for experimentation in PCI.

The work of Zirkle and Bloom (9), utilizing a 2 Mev Van de Graaf electrostatic accelerator, has dominated PCI during the past decade. Two methods were used: a crossed-slit apparatus and thin metal foils punctured with a sharp needle to collimate low energy protons. The former produced a poor geometric distribution because of the long path of travel for low energy protons between the collimator and the target. The thin metal foil type of collimator was ideal for low energy protons, and was used in PCI. The alignment system consisted of a carefully machined assembly which reproduced cell positions under the Van de Graaf, or under the microscope. It was not possible to monitor directly the number of particles which passed through the cell, because of the high attenuation of 2 Mev protons in materials. The proton flux during PCI was determined by first measuring the flux without the target material present for a fixed period of time and then irradiating the target material for a similar fixed period of time. This, of course, assumed the flux to be constant over the times of interest. Despite the complications of this procedure, several cells were irradiated and results were evaluated.

Discovery of the Van Allen belts with their high radiation fields, largely protons, has provided the impetus for new investigations into a field which has already proved useful in evaluating radiation damage. The technics and apparatus described herein have also proved useful in microsurgical evaluation. We report the development of an apparatus for PCI with 16 Mev protons which is believed to be the logical extension of the work of the earlier investigators.

2. THEORY OF PROTON PCI

Except for the unproved possibility of electrically or magnetically focusing a proton beam into a small area, proton collimation consists of shielding all protons except those

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falling on a selected small area. The thickness of the shielding must be sufficient to completely stop unwanted protons. A channel of some type is provided in this shielding so that a few protons, which constitute a microbeam, are allowed to pass through. If none of the protons in the microbeam interact or lose energy before striking the cell culture chamber, and if the initial microbeam is monoenergetic, the microbeam will also be monoenergetic.

With suitable alignment apparatus, a cell can be positioned in the microbeam for PCI. The remaining tasks are to determine the number of protons which pass through the cell, and to determine the exact part of the cell hit by each proton. The results of a complete experiment, modeled after the ideal conditions described above, could prove highly useful.

3. DEVELOPMENTS AT T.N.C.

Numerous problems are involved in converting these principles into a workable apparatus. The main ones concern the development of (1) a high yield reaction chamber, (2) a suitable collimation device, (3) alignment technics, (4) a system for monitoring the dose delivered to tissue culture cells, and (5) a tissue culture chamber. These problems will be considered separately and are covered below.

He^3 (d, p)He^4 reaction chamber

It was decided to use the $He^3(d,p)He^4$ reaction as the proton source. The resonance region for this reaction is 400 to 460 Kev deuteron energy (8). Proton yield is enhanced in the resonance region (2). At this deuteron energy the protons in the 0° position have a 16 Mev energy. This energy decreases to 14 Mev as one rotates to the 90° position. Since only the protons at the 0° position are used in PCI, this variation in energy does not present a problem.

Deuterons for the $He^3(d,p)He^4$ reaction are obtained from a 2 Mev electrostatic accelerator. The deuteron beam is magnetically analyzed and electrostatically focused. The resultant deuteron beam enters a 0.125-inch diameter He^3 gas reaction chamber. Prior to use, the He^3 gas is purified in a charcoal trap filter at −195° C. and is repurified periodically.

Since part of the deuteron energy is lost going through the entrance foil of the reaction chamber, the energy of the incident deuteron beam is varied from 1.0 to 1.6 Mev, depending on the type of gas chamber used and the thickness of the entrance foil which separates the He^3 gas from the accelerator vacuum. For maximum proton yield the center of the nuclear reaction should occur at the center of the reaction chamber. Since the yield of the reaction is also a function of the number of incident deuterons, and the volume of He^3 gas, obviously a higher deuteron beam current and a greater He^3 gas pressure will increase the proton production. Figure 1 displays such a reaction chamber, fitted with molybdenum foil 0.0002 inch thick. This foil is sufficient to withstand the high deuteron beam current and high gas pressures necessary for maximum proton yield. With this configuration and with 2 atmospheres of He^3 gas, the maximum proton yield was obtained at a deuteron bombardment energy of 1.5 Mev. The protons exit at $\theta = 0°$ through a 0.0005 inch thick tantalum foil which stops any deuterons up to 2 Mev and only absorbs about 0.25 Mev of the 16.35 Mev proton energy. This reaction chamber is currently in use in partial cell irradiation and in collimation work.

The reaction chamber is constructed of a large piece of brass 4 inches in diameter. Two purposes are served by the large size: (1) it supports several other pieces of equipment; and (2) the large mass helps to absorb the heat created by the high energy deuteron beam. (Here it is noted that 5 μa. of deuteron beam at 1.5 Mev dissipates 7.5 watts of power in the reaction assembly.)

The proton energy from this reaction (16.35 Mev at $\theta = 0°$) is well known (1). Figure 2 illustrates an experimentally determined energy distribution of the protons from this reaction. As can be seen from the drawing, the proton energy peaks at about 16 Mev with a 0.4 Mev energy spread. This illustrates the monoenergetic yield of this reaction.

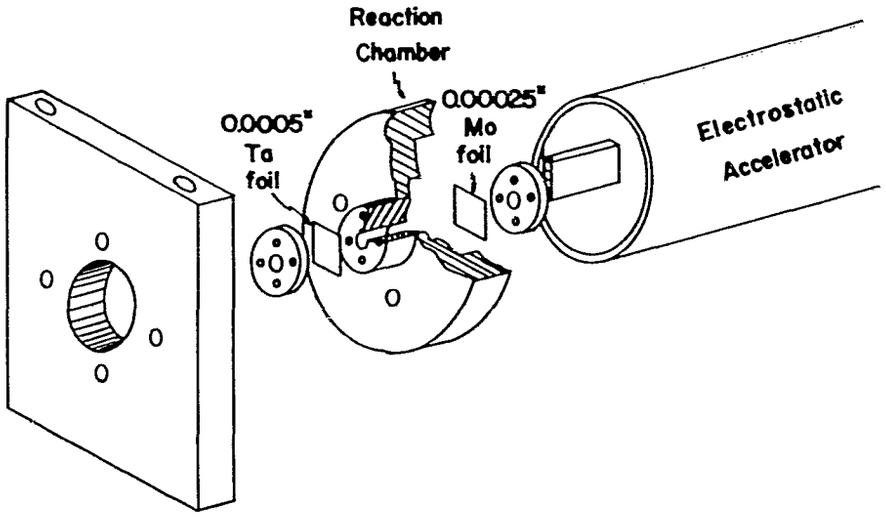

FIGURE 1

Cutaway drawing of $He^3(d,p)He^4$ reaction chamber used in single proton irradiations.

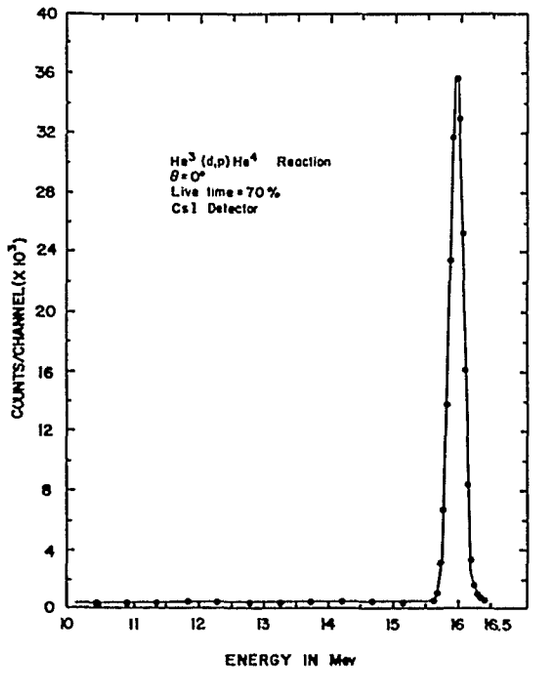

FIGURE 2

Pulse height distribution of 16 Mev protons.

Assuming a deuteron beam current of 1 μa. and He^3 gas pressure of 1 atmosphere, theoretic calculations indicate a proton yield of 1.2×10^7 protons/second. Experimental determination of the proton yield in the 0° position under these conditions resulted in a value of 3.5×10^7 protons/second. The higher experimental value for proton flux may be attributed in part to favorable geometry conditions at $\theta = 0°$. Furthermore, the reaction in question constitutes an extended source, rather than a point source, which further increases the proton yield at $\theta = 0°$.

Gage block and foil collimation apparatus

PCI requires a microbeam of only several microns diameter. Since it is a practical impossibility to produce such a tiny, yet well-defined hole in any material which is thick enough to stop a 16 Mev proton, it was decided to use a "gage block and foil" apparatus for the collimator. In this method of collimation, precision, polished steel, machinist gage blocks are employed which possess two parallel surfaces, flat to 1 millionth of an inch. Between two

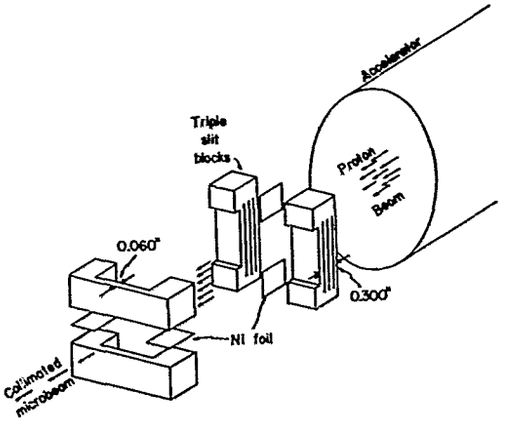

FIGURE 3

Gage block and foil collimator.

such blocks, thin, high quality, metal foils are inserted, which produce a separation of the blocks of about the thickness of the foil. An identical set of blocks, similarly separated, may be placed just above and at right angles to the first set of blocks. Hence, the perpendicular slits create a small collimation hole (see figure 3).

Nickel foils are available in thicknesses down to 0.2 μ. To control the size of the collimator aperture, different thicknesses of foil are used. For maximum collimation the foils may be removed completely. Several different gage block shapes have been investigated in order to determine the optimum configuration for collimation. The basic gage block is shown in figure 4a. This type of gage block configuration produces a very narrow ribbon

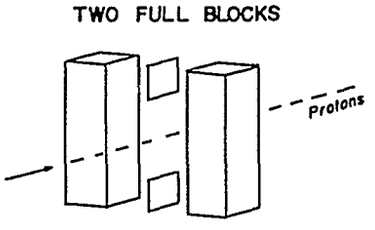

Figure 4a

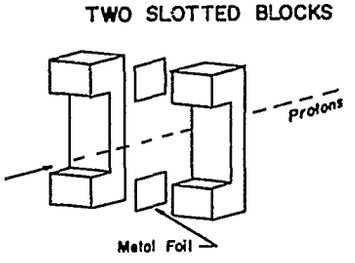

Figure 4b

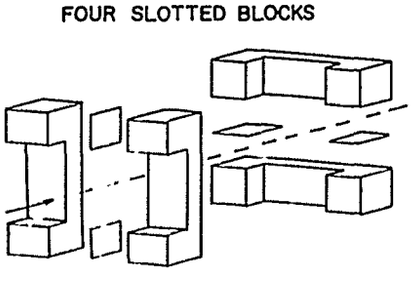

Figure 4c

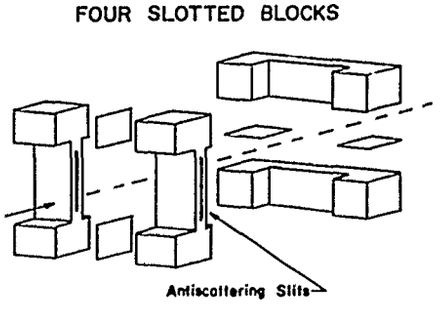

Figure 4d

FIGURE 4

Different gage block shapes.

of proton tracks on nuclear emulsion plates. The long path of proton travel (0.350 inch) through these blocks contributes to the best collimated proton distribution. To decrease the proton path, the gage blocks were specially machined so that the thickness was reduced to 0.100 inch. This type of gage block is shown in figure 4b. The increase in yield of collimated protons amounted to over 5 times that of the uncut gage blocks. The degree of collimation was reduced somewhat.

The addition of a second pair of gage blocks resulted in a configuration as shown in 4c, which produced some excellent collimation. Further experimentation, however, seemed necessary since the collimated proton yield often amounted to only one particle every 30 minutes.

The thickness of the blocks was further decreased to 0.050 inch in order to increase the yield. The first pair of gage blocks was constructed with a slit in their collimation faces which helped produce slightly better collimation.

To speed research, a higher yield (10^{12} protons/sec.) of low energy (1 Mev) protons was obtained from the primary accelerator proton beam by bypassing the $He^3(d,p)He^4$ reaction. By use of a gage block configuration as shown in figure 4d, excellent collimation of these particles was obtained. In some instances the geometric distribution of proton tracks was about 1 by 5 μ in area and was consistently 2 by 10 μ or less in area. The longer spread in one direction is caused by divergence of the beam from that part of the total collimation produced by the first pair of blocks. At the exit of each pair of blocks, the proton microbeam approaches a width which is equal to the thickness of the foil used. Examination of nuclear emulsion plates placed directly against the exit of the gage blocks reveals a number of protons scattered at wide angles from the main part of the proton track distribution. These tracks result from protons scattered through the corners of the steel gage blocks. This scattering cannot be completely eliminated, particularly when using 16 Mev protons, but it may be minimized by proper alignment of the gage blocks with respect to the $He^3(d,p)He^4$ reaction, and by clean surfaces on the gage blocks.

An improvement in collimation effected by the first or lower pair of gage blocks has been accomplished by cutting three slits into the collimation face of the lower pair of blocks, as shown in figure 5. This design takes ad-

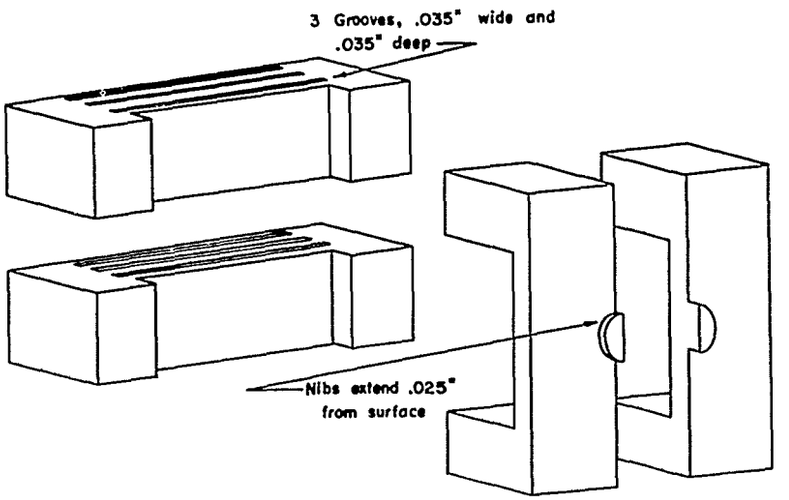

FIGURE 5

"Triple slit" and "nib" blocks in position for full collimation.

vantage of the wide difference in proton ranges for steel and for air (i.e., 0.8 mm. for Fe and 280 cm. for air). A type of antiscattering system has been introduced which reduces the beam divergence from the first pair of blocks and helps to "hold" the collimation for a greater distance. Since the nuclear emulsions and the target biologic cells are placed directly against the exit of the second pair of blocks, the block size is adjusted for maximum yield rather than for maximum collimation. This means that the thickness of the second pair of blocks is reduced to 0.060 inch, which is sufficient to stop 16 Mev protons. This configuration, shown in figure 3, is in use for partial cell irradiation with 16 Mev protons.

The requirements of a biologic culture chamber have dictated a further modification of the second pair of gage blocks. It became necessary to add a protrusion, 0.025 inch in height, to the exit surface of the second pair of blocks. Surface irregularities are determined by examining the block surfaces under an optical flat. The curving of the interference fringes denotes irregularities. A curving equal to the distance between two interference reflections corresponds to ½ wavelength or 11.6 millionths of an inch in helium light. Curvature of the interference fringes indicates highs and lows on the block surfaces. Only those blocks that are flat to within 6 millionths of an inch have been found successful for use in proton collimation. Those showing greater irregularities cannot be used and are discarded.

For maximum proton yield through the collimator it is necessary that the "channel" formed by the collimator be aligned with the center of the reaction chamber. This presents a free path of travel to the largest number of protons. This alignment is accomplished by mounting the gage blocks on a flat machined steel surface which is perpendicular to the exit port of the reaction chamber. In addition, the blocks are mounted against a fixed 90° gage block to insure that the collimating surfaces are perpendicular to one another and to the machined steel surface, and thus parallel to the travel of the protons.

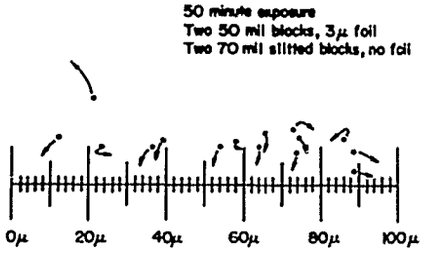

Figure 6

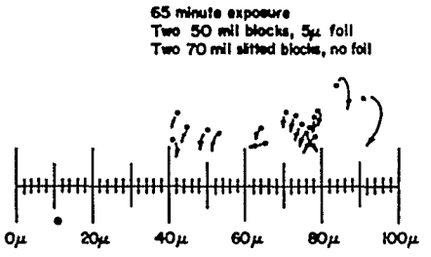

Figure 7

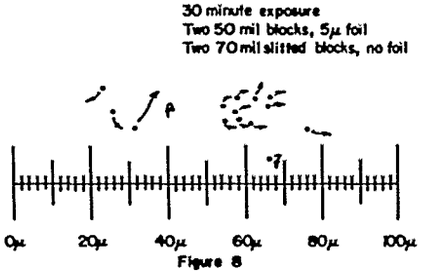

Figure 8

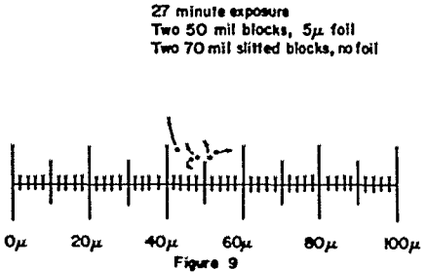

Figure 9

FIGURES 6 to 9

Proton distribution on nuclear emulsions.

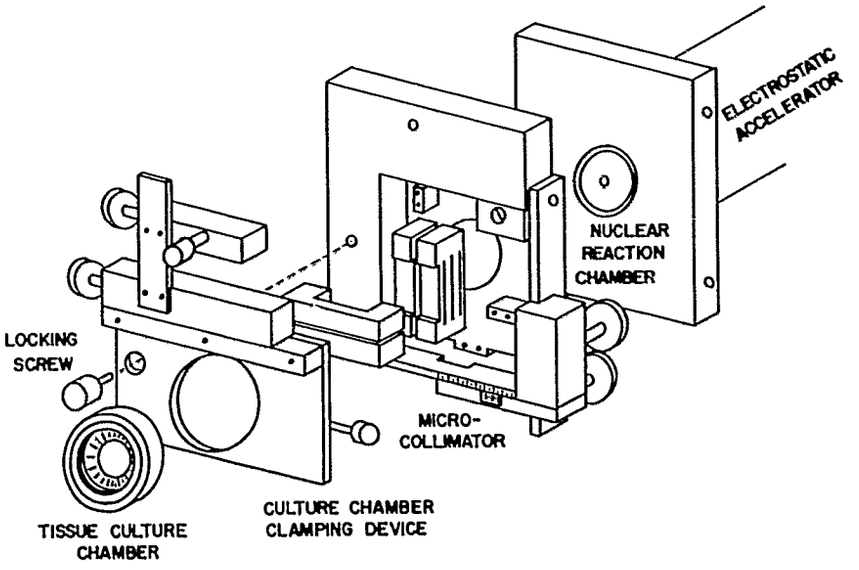

FIGURE 10

Tissue culture chamber and alignment device.

The degree of proton collimation is measured by recording proton tracks on Ilford nuclear emulsions. These plates are placed directly against the proton exit surface of the gage blocks. The plates are unwrapped and the emulsion makes contact with the blocks. Figures 6, 7, 8, and 9 are representative of the proton distribution seen on some of the nuclear emulsions. The differences in these proton distributions were probably caused by slight variations in the alignment of the collimation channel with the center of the reaction. This is another illustration of the precision in technics required to align the collimator properly. Small distributions are shown in figures 8 and 9 and are used in partial cell irradiations.

Alignment system

The cell alignment device is designed to position the microaperture formed by the gage blocks before a source of high energy protons and to position a biologic cell at the exit of this microaperture. This system is basically a set of two mechanically connected microscope mechanical stages (fig. 10). The modified Bausch and Lomb stage aligns the microaperture before the most intense portion of the $He^3(d,p)He^4$ reaction and aligns the microaperture with an ocular eyepiece cross hair. The latter operation helps insure correct visual placement of the tissue culture cell (target cell) with the microaperture. The modified Unitron stage moves the cell chamber, and hence the target cell, in relation to the microaperture.

The following procedure is used to align the tissue culture cell in the proper position for proton bombardment. The modified Bausch and Lomb stage is clamped in place on a metallurgic microscope stage. One pair of gage blocks is locked into position on the Bausch and Lomb stage. This forms the vertical slit of the collimator which is moved into place under the vertical hairline of the ocular eyepiece. Then the second pair of blocks is clamped in place above the first set. This forms the horizontal slit of the collimator. The entire collimator is then moved in the vertical direction until this horizontal slit falls under the horizontal hairline of the ocular eyepiece. The top blocks are then removed momentarily to recheck the alignment of the bottom set of blocks. This procedure insures that the microaperture "channel" lies directly beneath the intersection of the cross hairs.

Next, the modified Unitron stage is installed in position on the Bausch and Lomb stage. The cell chamber is clamped into place on this stage. The Unitron stage has vernier adjustments which allow it to be moved with respect to the Bausch and Lomb stage. The target cell is positioned under the cross hairs and the Unitron stage is then locked to the Bausch and Lomb stage. This fixes the target cell directly under the microaperture "channel."

The entire assembly is then removed from the metallurgic microscope stage and installed in place in front of the reaction chamber on the accelerator. The assembly is then adjusted to a predetermined position which aligns the collimator "channel" with the center of the reaction chamber. The cell is now in position for bombardment.

Following bombardment the assembly is removed from the accelerator and again clamped in place on the metallurgic microscope stage. The target cell is then placed in its original position under the cross hairs of the ocular eyepiece so that the alignment can be checked. During the time this assembly has been in use, this alignment has held for all experiments.

Monitoring technics

The monitoring system used in PCI is shown in figure 11. It consists of a shielded CsI crystal coupled to an RCA 6342 photomultiplier tube. The output pulses of the photomultiplier tube are fed into a linear amplifier. The amplified signal is fed through a coincident circuit to a multichannel analyzer which determines the proton energy after it has passed through the target cell. The amplified signal is also fed to a scaler which records the total number of protons that have passed through the cell.

The CsI detector may be positioned directly behind each cell chamber during irradiation. It is then possible to record the energies of the protons which have passed through the cell. This is, of course, one of the main advantages gained by utilizing high energy protons for PCI. About 10% of the collimated protons do not have sufficient energy to reach the CsI crystal and a correction factor for this effect is determined, where possible.

Figure 12 is a drawing of a typical 16 Mev proton spectrum. The open circles indicate collimated protons which have passed through the collimator, the target biologic cell, and the sustaining fluid surrounding the cells. The prominent uncollimated 16 Mev proton peak establishes a reference point for determining the energy of each recorded proton. Detection of protons does not occur below the point marked "bias," which is usually set at a proton energy of 6 Mev. It has been found that less than 5% of the collimated protons have energies in this region. On the other hand, the 6 Mev bias setting is sufficiently high to remove nearly all counts caused by other types of irradiation such as low energy gamma rays and neutrons. Several background counts for

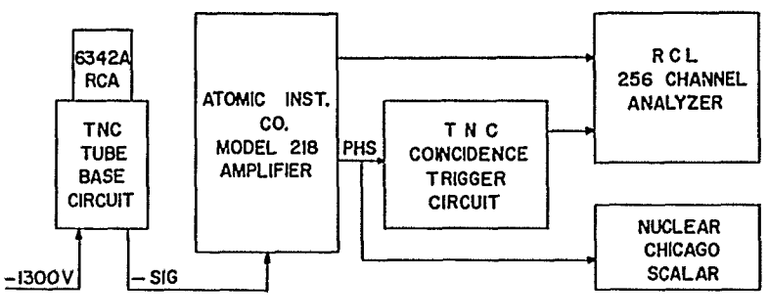

FIGURE 11

Electronic block diagram.

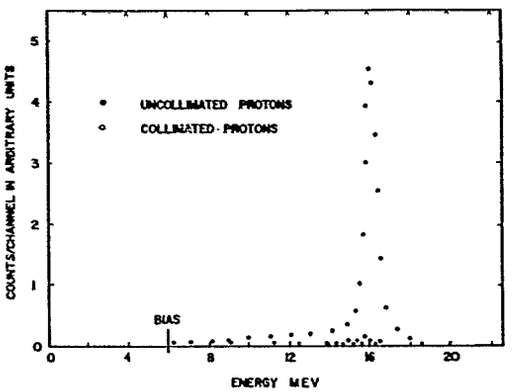

FIGURE 12

Energy spectrum obtained during collimation experiment.

periods in excess of 12 hours have not produced more than 2 counts above the 6 Mev bias per run.

In order to extrapolate for the incident proton energy on a biologic target, the energy loss curves have been calculated so that the incident energy may be read directly from the energy recorded in the pulse height analyzer (see figure 13).

Tissue culture chambers

The requirements for the design of tissue culture chambers used in partial cell irradiation are very exacting. From the viewpoint of the physicist conducting PCI, it is essential that the cell chambers meet the following requirements:

a. The target biologic cells should be within about 10 μ of the exit of the collimator for maximum collimation effect.

b. The tissue culture chamber must be thin enough so that the protons can pass completely through the medium and thus be recorded. The maximum allowed thickness is 1.5 mm.

c. Since the cell position is viewed through the metallurgic microscope during the alignment process, the chamber thickness must be less than the working distance of the objective lens being used. These working distances are 14.2, 2.8, 1.8, and 0.91 mm. The last objective lens which is a 40× B and L, is seldom used because of this problem.

d. It is desirable to have a round tissue culture chamber so that it may be rotated as a means of providing more possibilities for cell bombardment.

From the viewpoint of the biologist, it is essential that the chambers meet the following requirements:

a. The chamber must be of sufficient volume to contain adequate sustaining fluid necessary for cell survival. (It is noted that the ordinary Rose chamber used in much biologic work has an interior volume of 1.6 cc.)

b. The materials used in construction must be nontoxic to the cell culture being used. Excellent materials are glass, mica, and silicon gasket.

c. The chamber should be easily assembled and disassembled and should have a place for inserting hypodermic needles, which may be used to remove and replace the fluid inside the chambers.

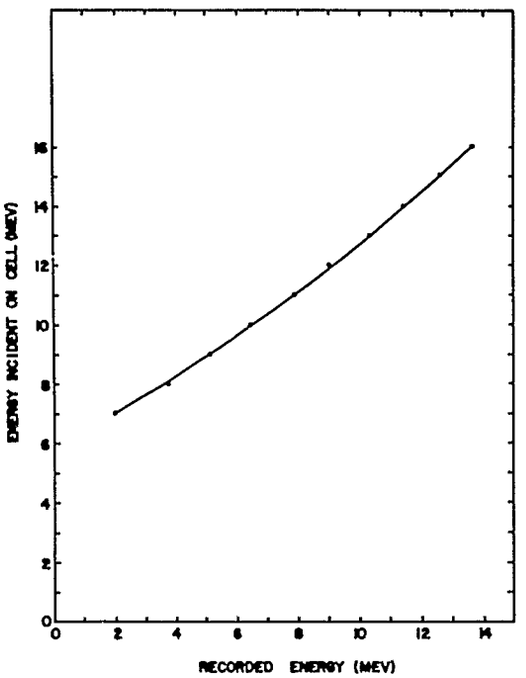

FIGURE 13

A typical energy loss curve for one of the chambers. The 1 mm. thickness of sustaining fluid absorbs most of the lost energy.

d. Since all microscope mechanical stages are designed to position rectangular objects of about 1.5 by 3.0 inches, a holder is necessary to provide a means of handling the round chamber on the microscope stage during postirradiation observations.

The two lists of requirements just presented are not exactly compatible, and it became necessary to compromise both viewpoints. A change in the gage block collimator design was necessary. The requirement that the biologic cell be positioned right at the collimator exit was maintained, but the shape of the second pair of gage blocks was machined with a small protrusion on its upper surface (see figure 5). This protrusion is machined to a height of 0.025 ± 0.0005 inch, which is the thickness of the bottom lip of the new cell chambers which was designed to fit the protrusion. Figure 14 is a drawing showing the exact specifications for the construction of the metal parts of these new cell chambers. It may be noted, with reference to figure 15, that these new chambers are easily assembled since they screw together by use of a small spanner wrench as a tool. A nonrotating washer is installed just below the screw ring in order to prevent scratching the 50 μ mica window during the assembly.

Since all the fluid is sealed between the mica windows and a silicon gasket, the sustaining fluid in these chambers does not touch the metal walls of the container. All parts are easily removed for cleaning and sterilization. The thickness of the mica windows may also be varied according to the requirements of various experiments, and the thickness of the silicon gasket may be varied to change the cell chamber thickness and fluid volume. Silicon gaskets currently in use are 1.5 and 1.0 mm. thick, which give cell volumes of 0.75 and 0.50 cc., respectively. The average diameter of the interior of the chamber is 1 inch. Access for replacing the sustaining fluid is provided through lateral holes in the vertical walls of the chambers. These holes match the position of the silicon gasket.

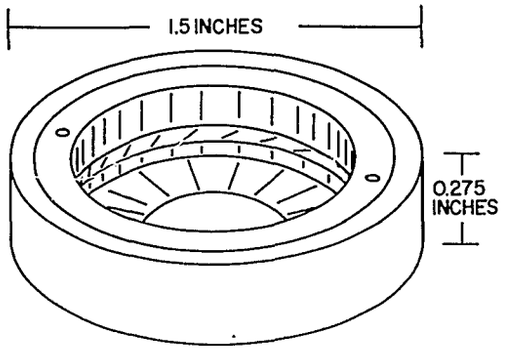

FIGURE 14

Tissue culture chamber.

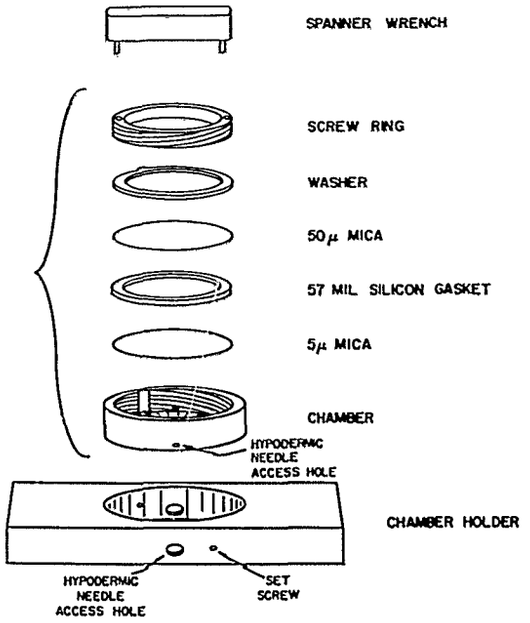

FIGURE 15

Unassembled cell chamber and chamber holder.

A rectangular chamber holder has been constructed for positioning the chamber in a microscope mechanical stage. The cell chamber is held securely in the chamber holder by two 2-56 Allen set screws. It should be noted, as shown in figure 15, that access for hypodermic needles is provided even when the chamber is in the holder. The chamber, however, must

be correctly placed for this operation. A narrow lip in the chamber holder prevents the chamber from slipping out. Tests of the pilot construction for these chambers indicate that there is no fluid leakage and that there is adequate access for the microscope objectives.

4. CONCLUSIONS

Protons from the $He^3(d,p)He^4$ reaction of 16 Mev energy are collimated by use of machinist gage blocks to form perpendicular slits. A means for aligning biologic cells in the resulting proton microbeam was devised which consists of a dual mechanical microscope stage positioning system. Specially constructed biologic cell chambers fit into this system. A scintillation detector monitors the protons emerging from the culture chamber. The energy spectrum of the emerging protons is obtained from a multichannel pulse height analyzer. The number and energy of the protons are obtained from this spectrum.

Two types of collimators are now operational. One type consists of 4 gage blocks which produce a proton distribution of approximately $2 \times 10\ \mu$ for 65% of the proton tracks. Although the yield of this type of collimation varies, it usually amounts to 1 proton per minute. A second type of collimation is that in which a long ribbon-shaped proton distribution is produced by use of only two gage blocks. A much higher yield of protons is obtained from this configuration and usually provides 1,000 protons per minute.

Now that the apparatus is operational, studies are underway to determine the effects of localized bombardment of living cells with 16 Mev protons. Results from these studies will provide an insight as to the mechanisms of cellular damage.

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